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Estimation of tenofovir's population pharmacokinetic parameters without reliable dosing histories and application to tracing dosing history using simulation strategies

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Abstract

The objectives of this analysis were to develop a population pharmacokinetic model (PPK) for tenofovir without using potentially unreliable patient reported dosing records and to retrace patient dosing history using pharmacokinetic simulations conditioned on protocol design constraints to assess patient adherence. MTN-001 is a multi-center, open label, 3 way cross over study comparing oral, vaginal and combination (oral and vaginal) administration of tenofovir in healthy women. It was reported that self-reported adherence in this study was high (94 %), but serum concentrations indicated only 64 % of participants used tablets consistently. A method based on superposition was applied to develop the population pharmacokinetic model considering only observable clinic visit dosing information. A two compartment model described the data well and the parameters agree with the literature reported values. Race was not a significant covariate on clearance. Retracing of the dosing history with 3-past dose resolution was not successful. Trial simulations with full adherence assumption predict a median C_{min} of 68 ng/ml, which is in agreement with literature reports. Non-adherence at 25% resulted in 37-51% reduction in C_{min} using one coin and two coin models, respectively. Population analyses should consider some method of correction for non-adherence to avoid biased estimates.

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Keywords

population pharmacokinetics; tenofovir; superposition; adherence

Introduction

Tenofovir disoproxil fumarate (TDF, Viread®) is currently approved for the treatment of HIV-1 infection in combination with other antiretroviral drugs¹ and is the first drug to be approved (300 mg, QD) for pre-exposure prophylaxis (PrEP) of HIV infection as part of a fixed dose combination with emtricitabine (Truvada®). TDF is an oral pro-drug that undergoes hydrolysis to form tenofovir (TFV) in plasma. TFV, in turn, is phosphorylated by intracellular kinases to form tenofovir diphosphate that is responsible for inhibition of viral replication. During the clinical development of oral tenofovir/emtricitabine for PrEP, the outcomes of several randomized controlled trials (RCT) varied greatly in HIV seroconversion outcome. For example, relative risk reduction ranged from 42% to 75% in the two studies submitted to FDA as part of the supplemental new drug application (sNDA) supporting tenofovir/emtricitabine for prevention. However, no statistically significant relative risk reduction was observed in two other large trials²⁻⁵. PrEP efficacy was highly correlated with adherence which is cited in the tenofovir/emtricitabine package insert⁶.

A quantitative understanding of TFV adherence patterns is vital to understand the causes of PrEP failure and, potentially, indicate the need for an adherence intervention during PrEP RCTs and in clinical practice. Several methods used for quantitation of adherence such as research participant diaries or recall, and pharmacy pill counts suffer from inaccuracy or easy falsification. Pharmacokinetically-based estimation of patient adherence was studied for anticancer therapies⁷ by decoding dosing information based on *a priori* pharmacokinetic modeling, but variability makes it perform poorly in estimating adherence.

MTN-001 (ClinicalTrials.gov Identifier: NCT00592124) was a Phase 2 open label crossover study of the pharmacokinetics, adherence, and acceptability of daily TDF 300 mg tablet taken orally and tenofovir 1 % gel used vaginally^{8, 9}. In MTN-001, self-reported adherence for all regimens was high (94%), but serum tenofovir concentrations indicated that only 64 % of participants used tablets consistently based on expected TFV concentrations observed in other studies using conventional non-compartmental analyses⁹. We propose that a model based analysis approach can add value in interpretation of such data. However, population pharmacokinetic analysis is also sensitive to the impact of poor adherence given the common assumption of full adherence and accurate dosing time data, neither of which are reasonable assumptions in the case of MTN-001 (and, likely, numerous other studies) without the use of directly observed therapy (DOT). This poses significant challenges to achieving unbiased parameter estimates¹⁰⁻¹³. Several approaches have been investigated to handle this situation by including medication event monitoring systems (MEMS) based dosing histories^{14, 15}, a mixture modeling approach¹⁶, a Bayesian approach¹⁷, a formalism of PK model including the stochastic drug intake behavior of patients¹⁸, and a missing dose method¹³. Gupta, *et al.*, systematically evaluated the missing dose method and proposed an alternative method as a way to dissociate the functional dependence of clearance estimation

and pre-dose concentration arising from an unknown or unreliable dosing history¹⁹. We describe the application of the method of Gupta to the MTN-001 data.

The objectives of this analysis are 1) to estimate population pharmacokinetic parameters of tenofovir in women after oral administration of 300 mg of TDF without the assumption of complete adherence or the use of patient-reported prior dosing information, and 2) attempt to trace the past dosing history of subjects using pharmacokinetic simulation methods.

Methods

Study design

Study Design and Research Participants—MTN-001 is a multi-center, open label, 3 way cross over study comparing oral, vaginal and combination (oral and vaginal) administration of tenofovir in healthy women. Eligible participants were sexually-active, non-pregnant women aged 18–45 years, using an effective method of contraception as described in the full protocol, (available at www.mtnstopshiv.org).and the clinical results of this trial are reported elsewhere^{8,9}. Briefly, each of 3 study periods is 6 weeks long and followed by a one-week washout between periods. A different TFV formulation or combination of formulations was prescribed for each of these periods in randomized sequence: oral 300 mg TDF, vaginal 1% TFV gel, and a combination of both oral and vaginal formulations. At the end of each 6 week period, blood, peripheral blood mononuclear cells (PBMC), vaginal tissue, cervicovaginal fluid, endocervical cells, and rectal fluid were collected with sample type and number of samples depending on site capacity. A pre-dose sample was also collected at middle-of-period (3-week) research clinic visits. The study had 7 participating clinical sites (2 in South Africa, 1 in Uganda, 4 in the US). For modeling purposes, we used only the plasma TFV concentration at the end-of-period (6 week) visit of the oral administration period where both pre-dose and post dose concentrations were available. The cohort of subjects with oral administration was instructed to take 300 mg of TDF every 24 hours for 6 weeks. Plasma samples were collected at the end of each period at pre-dose, 1,2,4,6 and 8 hours in US sites and a pre-dose and a random sample (1-7 hours post dose) in the African sites. The plasma samples were analyzed using a validated UPLC-MS/MS method (lower limit of quantitation = 0.3 ng/mL) as described elsewhere⁹.

Model building—The plasma concentration data was natural log transformed and analyzed using the nonlinear mixed effect modeling software NONMEM (version 7.2, ICON Development Solutions, Ellicott City, MD). WFN (Wings for NONMEM) and PSN (Perl Speak NONMEM) were used as interfaces for running NONMEM. XPOSE4 and R version 13.1 scripts were used for data plotting. The actual sampling times rather than protocol defined nominal times were used in the analysis.

Mathematical formulation of the method of Gupta is described in detail for a one-compartment model¹⁹. We applied and extended the methodology to a two compartment model with an assumption that pre-dose concentrations were collected in the post absorption, post distribution phase and during the β -elimination phase. Pre-dose

concentration was temporally linked to the proximal concentrations by time and the following model was fit to the data:

$$C = \left[SDF + C_0 e^{-\beta t} \right]$$

where C represents the plasma concentration and SDF is the single dose function given by the equation below:

$$SDF = \text{Dose} * \left(A * e^{(-\alpha * t)} + B * e^{(-\beta * t)} - (A+B) * e^{-ka * t} \right)$$

The model was parameterized in clearance and volume parameters by defining A, B, α and β , according to the classical two compartmental equations. C_0 represents the concentration present in the system at the time of the most recent dose and temporally proximal PK observations are linked to C_0 by virtue of time relative to the latest dose¹⁹.

Inter subject variability in the pharmacokinetic parameters was estimated using an exponential error model as follows:

$$P_i = TVP \exp(\eta_i)$$

Where η_i is the proportional difference between the hypothetical true value of the parameter estimate in the i th subject (P_i) and the typical population parameter value (TVP) and is assumed to be normally distributed with a mean of 0 and a variance of ω^2 . The residual error (including model misspecification and intrasubject variability, as well as errors in dosing, sampling times, and sample analysis) was evaluated using a log-transformed exponential error model as described below:

$$\ln Y_{\text{obs}} = \ln Y_{\text{pred}} + \varepsilon$$

Where, Y_{obs} is the observed plasma concentration, Y_{pred} is the model-predicted plasma concentration, and ε is a normally distributed parameter with mean of 0 and variance of σ^2 .

The likelihood ratio test for comparing hierarchical models where a decrease in NONMEM objective function (-2 LL) of 3.84 points was necessary to consider the improvement in model performance statistically significant ($\alpha=0.05$ and 1 df). Other selection criterion included improvement in goodness of fit and residual plots, increased precision in parameter estimation, parameter correlations, condition number less than 1000, and biological plausibility of the parameters.

Model qualification—The final model was qualified using (1) bootstrapping, (2) visual predictive check, and (3) comparison to the tenofovir pharmacokinetic data (compartmental and non-compartmental analyses data) available in the public domain. Bootstrapping ($n=2,000$) was conducted using PSN and was stratified by the clinical site to conserve the percentage of the subjects having extensive and sparse sampling. Visual predictive check

included 1000 simulations of the population pharmacokinetic model and observed data was overlaid to check the agreement between observed and different prediction intervals of simulated data.

Effect of adherence on the pre-dose concentration—Parameters from the population pharmacokinetic model were used to simulate the effect of adherence (defined as missing a dose entirely, not the error time of dose taking) with the built-in adherence models (one and two coin) in Trial Simulator (version 2.2. Pharsight Corporation, Thousand Oaks, CA).

The one coin adherence model assumes that, at each dose, there is a chance of non-adherence independent of adherence to the previous dose. For this set of simulations, 500 studies of 100 patients getting 300 mg QD of tenofovir were simulated, with average non-adherence of 0-25% assumed.

The two coin adherence model assumes a conditional probability of the i th dose based on adherence or non-adherence to the $i-1$ dose. Assuming that at the start of the simulation, each “patient” is in an adherent state,

$$P_{\text{miss}} = P(\text{non-adherence at Dose}_i | \text{adherent Dose}_{i-1})$$

is the probability that the patient will miss the i th dose, given that the previous days' dose was taken. Once the patient is in a non-adherent state, the probability that she will revert to the adherent state is governed by

$$P_{\text{take}} = P(\text{adherence at Dose}_i | \text{non-adherent Dose}_{i-1})$$

which is the probability that each patient will revert to the adherent state at the time of the i th dose, given non-adherence at the $i-1$ dose. For these simulations, P_{miss} was set at 0-25% as with the one-coin simulations. P_{take} was then set at $1-2 \times P_{\text{miss}}$, similar to what was reported in a MEMS monitored adherence study of zidovudine dosing in an HIV treatment setting²⁰.

Tracing dosing history by simulation—Decoding the prior dose history was attempted using a Bayesian tracing process as described by Li and Nekka¹⁸. We adapted this general approach, but did not use the true Bayesian decision process. It was assumed that all individuals were at steady state having followed the nominal dosing times (300 mg QD) as per protocol until the third dose prior to the clinic visit (shown in Figure 1, a minimally-influential assumption given the relatively short 17 hour plasma TFV half-life and low accumulation coefficient with daily dosing); different combinations of missing doses in the last three dose sequence were then simulated. This assumption gives a total of $2^3=8$ dose taking sequences –: 1-1-1; 1-1-0; 1-0-0; 0-0-0; 0-1-1; 0-0-1; 0-1-0, and 1-0-1 – where 1 and 0 represent taking a dose and not taking a dose, respectively. Thus, 1-1-1 represents full compliance, 0-0-0 represents full non-compliance and 1-0-0 represents missing last two doses prior to the clinic visit. It is also assumed that these are the exhaustive options of dose

taking in that period. Using empirical Bayesian estimates of individual pharmacokinetic parameters calculated as part of population pharmacokinetic model building, plasma concentration profiles were simulated for each individual as per the eight sequences listed above. We did not use C_0 estimate in these simulations and thus the simulated concentrations are a function of adherence-corrected parameters. The protocol instructs the dose to be taken prior to the longest period of rest with the clinic visit scheduled the next day which makes the pre-dose concentration at the clinic visit (generally occurring before noon) not a true 24 hour trough concentration. Based on the typical patient reported dose taking times, it was assumed to be a 12 hour nominal time post dose sample. Thus, the simulated plasma concentration (12 hour post steady state dose) in each individual resulting from each of the eight scenarios was compared with the observed pre-dose concentration at the clinic visit (Figure 1). All the scenarios with simulated concentrations within 60-140% of the observed pre-dose concentrations were selected in the first stage and the most likely 3-dose scenario (the scenario with ratio of observed to predicted closest in value to 1) is selected in the second stage. The limits were selected based on a normal distribution assumption with 20% coefficient of variation (residual variability estimated from the model) such that the ratio between the lower 5th and 95th percentile and mean will fall between 0.6 and 1.4.

Results

Of the 144 women who were evaluable in the primary study, plasma concentration data were available from 141 women who completed the oral administration cohort. Of these, 41 were excluded from the analysis, either due to having only one concentration available for analysis (N=31) or TFV concentration below the limits of assay quantitation (BQL, N=10). All 41 subjects excluded due to missing or BQL data were from African sites (where only two samples were collected at the clinic visit per protocol), therefore, one missing sample rendered the other sample useless for modeling using Gupta's method because at least one pre-dose concentration must be available for pairing with an observed post-dose concentration. Imputation of the BQL value (0.3 ng/ml) resulted in unacceptable standard errors (>100%) on the model parameters. The final dataset used for modeling included 100 subjects with 474 observation records. The subjects included in the analysis are described as follows: race, 32 white, 59 black/African-American, 9 other; mean body weight 77 (range 43-146) kg; mean age 31 (range 18-45) years; mean creatinine clearance 126 (range 69-259) mL/min (method of Cockcroft and Gault). The dose for tenofovir was adjusted to 136 mg based on the molecular weight ratio of the prodrug tenofovir disoproxil fumarate and tenofovir.

Model Building and qualification

Initial attempts to analyze the data with the full compliance assumption with steady-state option in NONMEM were not successful when using patient reported dosing data prior to the observed clinic visit dose. Gupta's method¹⁹ provided successful model convergence and covariance steps in NONMEM. A two compartment model with first order absorption and absorption lag was selected as the base model and resulted in a 160 point drop in NONMEM objective function value compared to a one compartment model. Between subject variability on inter-compartmental clearance was not retained because it did not provide significant

improvement in the objective function value or diagnostic plots. The pre-dose concentration is linked to the remaining profile temporally by time relative to the observed dose in the research clinic. This successfully dissociated the dependence of estimation of the clearance parameter from the pre-dose concentration. There was no correlation observed between the parameters C_0 and clearance, thus, confirming this dissociation. Population pharmacokinetic parameter estimates are given in Table 2 along with the bootstrap results. There were no trends observed in the basic goodness of fit plots (Figure 2). Visual predictive check (Figure 3) showed the 5th and 95th percentiles and the median of the simulated data from the model described the observed data well.

Covariates were evaluated by a forward addition and backward elimination process. Weight and race were shown as significant covariates on central volume and the adherence parameter (C_0), respectively, resulting in an additional 35 point drop in the objective function compared to the base model. However, the addition of weight as a covariate resulted in higher standard errors for the pharmacokinetic parameters and it explained only 1% of the between subject variability in volume of distribution of central compartment. C_0 is not a physiologically useful pharmacokinetic parameter and the covariate relationship will not be useful in clinical decisions. Race tested as covariate on clearance was not significant, in agreement with a published report in HIV-1 infected patients²¹. The comparison was done only for black compared to non-black races due to the small number of subjects (n=9) in the other race category. It must be acknowledged that the race variable is confounded with clinical site and further causal variables more complex in nature that includes biological, social and behavioral sources. The current model cannot separate these factors. Thus, no covariates were retained and the (simpler) base model is presented in this report. The final model showed no correlations between the parameters greater than 0.95 and the condition number calculated as the ratio of the highest to lowest eigenvalues was 365.

Comparison of Base Model to other Reports

The TFV pharmacokinetic parameter estimates for clearance, central volume, peripheral volume, and intercompartmental clearance in our MTN-001 analysis compared favorably to estimates from four other reports, falling within the range of values in those studies (Table 3). The notable difference was a much higher estimate for the absorption rate constant in our model. Our between subject variability (BSV) estimates fell among or below estimates in the other studies, again, with the exception of a higher value for our absorption rate constant BSV. All of the comparison studies were of HIV-infected patients, though Duwal, *et al*²², included a model based on two studies of healthy volunteers and one in patients. We also made comparisons to results of non-compartmental analyses from healthy volunteer studies from the literature in the later sections of this manuscript.

Tracing dosing history by simulation

Tracing of dosing history is inherently an ill-conditioned problem with many solutions and our attempt to decode the dosing history with 3 past dose resolution was not successful. Overlapping expected concentrations were observed for many individuals where the criterion makes only mathematical sense, but more than one scenario was practically possible. A total of 88 subjects out of 100 in this dataset had a traceable dosing sequence

within the 0.6-1.4 criterion and 51 subjects in the dataset have the full compliance (1-1-1) scenario within that criterion although it is not the most probable scenario in all of those cases. This makes the evaluation very liberal and gives the benefit of the doubt for assigning full compliance. This 51% full compliance estimate is lower than the evaluation done using a 40 ng/ml cut off value used by Minnis, *et al.*, in estimating adherence⁹. The difference between Minnis et al. and our approach was that Minnis et al. compared individual trough concentration to a population average concentration. In contrast, we set individual target concentrations depending on the simulations using individual pharmacokinetic parameters.

We illustrate this method in Table 4 with three individual examples. For individual #1, the expected concentrations based on all 8 adherence sequences and the observed pre-dose concentration show that the most likely adherence scenarios were 1-0-1, 1-1-1, 0-0-1, and 0-1-1. For additional comparison, individual #1's mid period (3 week) visit concentration was compared and it was reported as 106 ng/ml. Some individuals such as individual #2, did not result in any likely scenarios tested. The observed value of 277.9 ng/mL is consistent with C_{max} for oral TDF dosing and, therefore, most likely results from a white coat effect with a dose taken very soon before the clinic visit despite instructions to the contrary. The mid visit trough for individual #2 is BQL which is consistent with a "1-0-0" or "0-0-0" sequence probably indicating non-adherence. For individual 7, the most likely scenario was selected as "1-0-0" with a low concentration value of 5.4 ng/ml best explained by missing the last two doses. By contrast, her mid-period pre-dose TFV concentration was 127.5 ng/ml which is closest to the 1-1-1 adherence scenario simulation, demonstrating heterogeneity in adherence across study visits. When we compared the concordance between predose concentrations for the end-of-period (6 week) visit and compared them to the middle-of-period (3 week) visit, we identified 38% of subjects having greater than 50% difference between those concentrations within an individual, again, indicating heterogeneity in individual adherence given qualitatively similar expected time post last dose.

Simulation of effect of adherence on the C_{min} of tenofovir

Effect of non-adherence by one and two coin models on the C_{min} of tenofovir are shown in Figure 4. The two coin models predict a greater impact on the C_{min} compared to the one coin model and is an expected result.

Discussion

Previous reports on population pharmacokinetic analyses of tenofovir²¹⁻²⁹ used a two compartment model for describing pharmacokinetics of tenofovir in plasma. Population pharmacokinetic parameter estimates in this report are in agreement with the reported values from several other comparable populations. Tenofovir rate of absorption was not well characterized in our model due to lack of data in the absorption phase. Lag time inclusion in the model resulted in a significant drop in the NONMEM objective function and thus was retained. We did not opt for fixing a reported K_a value because this drug has a known food effect and rate of absorption could be different depending on the design of the study.

All the other population pharmacokinetic models in the Table 3 comparison were developed from HIV-1 infected patients (except the report from Duwal, *et al.*,²¹ which included mixed

healthy and infected subject studies), though tenofovir plasma pharmacokinetics was previously reported to be similar between healthy volunteers and patients³⁰. Steady state AUC_{0-24} calculated (3341 ng*hr/ml) from our current model-estimated clearance agrees with the mean steady-state $AUC_{(0-24)}$ values reported in healthy volunteer studies ranging from 2800 ng*hr/ml-3560 ng*hr/ml after daily 300 mg TDF oral administration^{31,32}. Agreement of our model parameters with literature reports implies that the other studies had more adherent populations because those models did not use any adjustment for non-adherence. We illustrate some of the factors in those studies that can give rise to better adherence. Our report closely matches Baheti, *et al.*, parameter estimates and that analysis used well controlled studies (ATN056 and 1427). ATN056 was a single center study and ATN1427 implemented telephone contact to the subjects for the 2 days preceding the intensive pharmacokinetic visit to ensure adherence to and appropriate timing of medication administration. Dumond, *et al.*,²³ reported an increase in clearance with multiple dosing and this may also be an effect of non-adherence where it was stated that datasets were constructed using the limited available adherence and timing data with a variable number of doses between visits for each subject. It is notable from this report that the clearance after a single dose matches well with other analyses (including ours). Taking together the different single and multiple dose period estimates may imply the accumulation function is misspecified by the erroneous dosing information used in modeling multiple dose data.

Trial simulations using coin toss models³³ were conducted to evaluate the effect different levels of adherence had on the C_{min} parameter at Day 28 after daily dose of 300 mg TDF. In these simulations, the estimated C_0 parameter from the model was not used and only the classic two compartment model parameters were used. Two models, one and two coin, were used for simulations. In contrast to the one coin model, the two coin model assumes missing one dose decreases the probability of taking the next dose. Thus, the effect of non-adherence on the pharmacokinetics will be higher in the two coin simulation and that is evident in our case (Figure 4). In a study prescribing thrice daily zidovudine and using MEMS data for adherence assessment, the odds of missing a dose increased nearly twofold if the previous dose has also been missed, or if an extra dose was taken at the previous nominal dose time, while the odds of taking an extra dose are increased nearly fourfold if the previous dose was missed²⁰. In our simulations, the probability of dose retaking in the two coin model (P_{retake}) was fixed as $1-2*P_{miss}$ to approximate this behavior. Trial simulations with a 100% adherence assumption predicted a 24 hour C_{min} median concentration of 68 ng/ml following oral administration of 300 mg TDF QD (Figure 4). This estimate falls within the 53-71 ng/mL range for steady-state C_{min} reported in brief Phase I studies of daily oral 300 mg TDF conducted in healthy volunteers where the 100% adherence assumption is reasonable^{31,32}. However, the assumed conditional probabilities in the two coin model are theoretical since no estimated probabilities are available at this time. Analysis of MEMS data from large PreP trials could potentially provide the estimates of these parameters (P_{miss} and P_{take}) to inform such simulations.

Within MTN-001, a 40% lower C_0 was estimated in black research participants when compared to white participants. C_0 is a non-parametric estimate¹⁹ of the pre-dose concentration which is influenced by both adherence and pharmacokinetics. C_0 has to be interpreted with care based on the expectation of pre-dose concentration given full

adherence. The expected pre-dose plasma concentration in MTN-001 was approximately 120 ng/ml based on simulation. Since the pre-dose TFV concentrations in white subjects are closer to this value than black subjects, this indicates a difference among these groups in timing of the dose prior to the end-of-period clinic visit. Since there are no differences in TFV plasma PK parameter estimates between racial groups, the difference in C_0 values indicates adherence differences between these populations. The magnitude of the difference in pre-dose concentrations between the two populations represents a difference of 21 hours in time of prior dose based on our model simulations. This elapsed time difference is far greater than the time of day variability that might otherwise account for some of the racial difference. These estimates are also conservative as the BLQ sample data were excluded and were more numerous among black research participants. Wang, *et al.*,³⁶ argued that the basic concept of steady state needs redefinition in case of non-compliance such as dose omissions and dosing time errors. They proposed that the pre-dose concentration behaves as a random variable rather than a constant value as assumed with classical steady state assumptions. Their statistical model predicted that the dose omissions cause a bimodal distribution in pre-dose concentrations whereas increased dispersion results from dose timing errors. Further methodological research is warranted to understand the statistical behavior of the C_0 parameter as a measure of adherence.

It is a tradeoff between sensitivity to detecting non-adherence events and the backward looking distance in history that these pharmacology-based adherence tracing methods can offer. In the case of tenofovir, having a half-life of ~17 hours allows us interpretation of TFV concentration data only within the last 2-3 days to quantitatively estimate adherence. It is not possible to decode the full dosing behavior, except for very short periods, due to the limitations in the vision that PK measures have looking back in history. Even so, brief or sustained non-adherence can cause serious biases in interpretation of the data with traditional pharmacokinetic data analysis. Some of the other biological specimens collected (PBMC, vaginal biopsy) in the study may provide an opportunity for a longer view into the history due their longer half-lives once we have a reliable pharmacokinetic model to describe their concentrations. Gupta's method was not applied to other specimen data because there were no data to support the linear pharmacokinetic behavior for PBMC concentrations of the phosphorylated metabolite or the sampling scheme was not applicable (single time point for biopsies).

Some of the weaknesses of the current model have to be highlighted. First, in contrast to the original methodology paper, the method is applied here for a two compartment model drug. It is assumed that the pre-dose concentration is in the post-absorption/post-distribution phase and declines at β -half-life. This assumption has some support from the protocol design that the subjects were instructed not to take any missed dose if the next dose was estimated to be due within 6 hours, thus in theory, represents β -elimination phase. However, some situations of non-compliance such as taking a dose just before the clinic visit (potential white coat effect) would potentially violate that assumption but we do not have a way to rule out such an occurrence in the model framework. Second, the sampling scheme was constrained to the duration of clinic hours after an observed dose and not prospectively designed using optimal sampling principles. Subjects from African sites have only two samples collected and this causes Bayesian shrinkage, but the covariate testing was done in a NONMEM environment

to avoid problems with shrinkage³⁵. However, the empirical Bayesian estimates used for simulations for tracing adherence will still have shrinkage. Third, we had to exclude subjects with missing samples whether due to only one sample available or a BQL sample value. We could not apply likelihood-based approaches³⁶ for handling BQL when the pre-dose concentration is BQL because of the fundamental feature of dissociation between estimation of clearance and predose concentration arising from an unknown dosing history. Exclusion of BQL data also creates a downward bias by excluding those individuals with the worst adherence. To further support our assumption that BQL in plasma tenofovir concentration was due to a missed dose, we checked for any BQL values that occurred at both the pre-dose and post-dose sample times and then examined that participant's concentration data from the vaginal and dual dosing period in an effort to use intra-subject variability as supportive evidence. Only three subjects had BQL concentrations both pre-dose and post-dose (2 from African sites, 1 from US site) with this situation. The first subject's oral dosing period had BQL in both pre-dose and post-dose samples but dual dosing period concentrations were in line with the expectation. The second subject had BQL values for pre-dose and post-dose concentrations for both oral and dual administration cohorts, but her vaginal dosing period concentration 6 hours post-dose was detectable and consistent with the cohort expectations. The third subject (US site) had BQL value for 8 hour post dose concentration for oral administration but for both the dual and vaginal dose periods that were in line with expected values. These within subject discrepancies in the concentration data suggest that the BQL observation was due to a missing dose. A limitation of our dose tracing is that the look-back period is only 3 days given the relatively short half-life of the drug. However, longer half-life analytes may not prove any better in dose tracing given the even more poorly constrained conditions looking back over more days. Finally, we only considered a dose omission scenario for non-adherence assessment while both time of dose taking and additional doses can contribute to the concentration difference with respect to time past last dose. In our study, patient reported adherence shows that mode of time post dose is around 12 hours but having an objective measure would be more reliable. Adherence tracing is inherently an ill-conditioned problem and a combination of pharmacokinetic modeling with time of event monitoring using MEMS-type devices remains a logical avenue for a better understanding of adherence and drug exposure patterns within clinical studies. Thus, this analysis is not considered a formal population pharmacokinetic analysis of the data, but is exploratory in nature and the model needs prospective validation in a clinical setting before being utilized for making clinical dosing decisions. One way to qualify the model would be to conduct a clinical study where the adherence was known (MEMS or directly observed therapy) along with pre- and post-dose concentration data such that observed adherence could be compared to adherence estimated by Gupta's method. The current model has the potential clinical application of estimating unbiased individual pharmacokinetic parameters from an unknown prior dosing history.

Conclusions

A population pharmacokinetic model for describing tenofovir concentrations after multiple oral doses was developed without using the patient reported dosing history which was necessary given the poor level of adherence and inaccurate dosing time data given by

research participants. The method, originally proposed by Gupta, *et al.*, allowed us to separate adherence effects from pharmacokinetics effects in the study. The resulting model parameters are similar to the previously reported values from more adherent populations. The recent individual dosing history was traced backward using pre-dose concentration and simulations based on individual pharmacokinetic parameters (empirical bayesian estimates) from our adherence-adjusted model. In these tracings, only 50% of research participants were fully adherent, having taken their last 3 doses, indicating significant levels of non-adherence in the study population. Expected values associated with different levels of adherence were also described using trial simulations. Our application of Gupta's method enabled us to isolate adherence from pharmacokinetics thus avoiding substantial adherence-related bias in the model-based estimates from our poorly adherent study population.

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Figure 1.

Illustration of assumptions in the process of tracing dosing history by simulations: three doses prior to the clinic visit (dotted lines) were appropriately omitted to create different non-adherence scenarios (adherence scenario in this figure represents 1-1-1, all three doses prior to the clinic visit were taken).

Figure 2.
Basic goodness of fit plots: DV-observed concentrations, PRED-population predictions, IPRED-individual predictions, CWRES-conditional weighted residuals, TIME-time in hours.

Figure 3. Visual predictive check plot (gray area represents 5th–95th percentiles of simulated data, solid line represents the median of simulated data, points represent observed concentrations).

Figure 4.
Effect of various levels of non-adherence on steady-state Cmin after QD administration simulated using one and two coin models in Trial Simulator®.

Table 1

Population Pharmacokinetic Parameters, Bootstrap Estimates and Comparison of the Current Model Parameters With Published Reports

Parameter	Estimate (%RSE)	Bootstrap estimates [median (95%CI)] ^a	Duwal et al (22)*	Baheti et al (21)	Jullien et al (29)	Dumond et al (23) ^ζ
CL/F (L/hr)	40.7 (17)	40.2 (8.6-102)	29.3	42.1	50.5	42.7 (SD) 71.3 (MD)
V _c /F (L)	293 (10)	298 (151-602)	244	224	297	187
V _p /F (L)	498 (23)	501 (295-922)	464	512	848	458
Q/F (L/hr)	137 (14)	136 (56-397)	71.4	175	80	158
K _a (hr ⁻¹)	7.65 (16)	8.81 (5.42-18)	1 Fixed	0.822	NE	1.05 (SD) 1.53 (MD)
T _{lag} (hr)	0.5 (0.3)	0.53 (0.50-0.80)	NA	NA	NA	NA
CO (ng/ml)	70.9 (8)	71.2 (60.7-82.0)	NA	NA	NA	NA
BSV CL (%CV)	62 (54)	60 (26-121)	NE	35	25	147 (SD) 73 (MD)
BSV V _c (%CV)	16 (46)	15 (6-23)	NE	79	60	47
BSV V _p (%CV)	44 (66)	42 (2-78)	NE	41	NE	83
BSV Q (%CV)	NE	NE	NE	NE	NE	158
BSV CO (%CV)	69 (24)	67 (52-83)	NA	NA	NA	NA
BSV K _a (%CV)	170 (24)	177 (134-239)	NE	NE	NE	69.8 (SD) 108 (MD)
Residual variability	20 (23) ^b	19 (15-24)	NE	18.3 ^b	41 ^c	10 ^b 34 ^c

NE- Not estimated

NA-Not applicable

Dumond *et al*- Data from HIV-infected pre-menopausal women in a single site study

Duwal *et al*- Data pooled from 3 independent single site clinical trials (two drug interaction studies in healthy volunteers and one Phase I/II study in HIV infected subjects)

Baheti *et al*-Data pooled from two single site clinical studies in HIV-infected subjects (ATN056, 1427)

Jullien *et al*- Data collected from HIV-infected patients monitored according to the plasma concentrations

* Model was parameterized in rate constants and CL/F was calculated as V_c/F*k_e, Q/F is calculated as V_c/F*k₁₂ and V_p/F is calculated as Q/F/k₂₁

^ζSD represents after single dose and MD represents after multiple dose

^an=2000, all the runs included irrespective of minimization status

^bAdditive error interpreted as SD with units ng/ml

^cProportional error (%CV)

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Table 2

Individual Examples of Dose Tracing Process by Simulation, Dose Sequence That Falls Within the Set Criteria is Shown in Bold Values

ID	Adherence Scenario	Simulated pre-dose concentration	Observed pre-dose concentration	Ratio	Probable adherence scenario
1	1-0-0	30	116	3.8	
1	1-0-1	128	116	0.9	*
1	1-1-0	72	116	1.6	
1	1-1-1	170	116	0.7	*
1	0-0-0	13	116	9.1	
1	0-0-1	111	116	1.0	*
1	0-1-0	54	116	2.1	
1	0-1-1	152	116	0.8	*
2	1-0-0	0	277.9	1841	None
2	1-0-1	30	277.9	9	None
2	1-1-0	2	277.9	127	None
2	1-1-1	32	277.9	9	None
2	0-0-0	0	277.9	26656	None
2	0-0-1	29	277.9	9	None
2	0-1-0	2	277.9	136	None
2	0-1-1	32	277.9	9	None
7	1-0-0	6	5.4	0.9	*
7	1-0-1	99	5.4	0.1	
7	1-1-0	27	5.4	0.2	
7	1-1-1	120	5.4	0.0	
7	0-0-0	1	5.4	3.8	
7	0-0-1	94	5.4	0.1	
7	0-1-0	23	5.4	0.2	
7	0-1-1	115	5.4	0.0	